

Vitamin B₁₂ in Activated Sewage Sludge¹

Sam R. Hoover, Lenore B. Jasewicz,
and Nandor Porges²

Eastern Regional Research Laboratory,³
Philadelphia, Pennsylvania

During studies of the biological oxidation of dairy wastes, it occurred to us that the mixed microflora might contain significant quantities of vitamin B₁₂. Further investigation confirmed this idea, and led us to test the vitamin B₁₂ content of aerator sludge from a municipal sewage treatment plant. The apparent vitamin B₁₂ content shown by direct assay with *Lactobacillus leichmannii* ATCC 4797 was determined essentially by the method of Skeggs, *et al.* (1). Since such activated sludge could be expected to contain almost any product of plant or animal metabolism, it was tested further by alkaline destruction of vitamin B₁₂ (2) and chromatographic separation on paper from other materials which stimulate the growth of the test organism (3).

The amount of vitamin B₁₂ indicated by the alkaline destruction procedure (Table 1, col. 4) was about that shown by chromatographic separation (not tabulated). Materials stimulating the growth of *L. leichmannii* which had *R_F* values of about 0.4, presumably thymidine, and 0.8 made up the major part of the components which move more rapidly than vitamin B₁₂ complex. Essentially the total activity shown by the direct assay was given by the sum of the various components separated chromatographically. These data indicate that the alkaline destruction method is a satisfactory measure of the vitamin B₁₂ activity of these preparations for *L. leichmannii*.

A vitamin B₁₂ content of 1.5 mg/lb (3.3 µg/g) has

¹ Report of a study made under the Research and Marketing Act of 1946.

² We wish to acknowledge the cooperation of J. C. Lewis, Western Regional Research Laboratory, in making the initial tests, and of Robert P. Bolenius, superintendent of the Abington Township (Pa.) Sewage Treatment Plant, for collecting experimental material.

³ One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

TABLE 1
VITAMIN B₁₂ CONTENT OF ACTIVATED SLUDGE
(All Data Calculated on Moisture-Free Basis)

1	2	3	4
Sample	Apparent B ₁₂ (µg/g)	Alkali- stable growth factors (as B ₁₂) (µg/g)	Vitamin B ₁₂ (by diff) (µg/g)
<i>Dairy waste sludge</i>			
Wet	8.8	1.5	7.3
<i>Municipal sewage sludge</i>			
Wet	9.7	0.4	9.3
Lyophilized	6.7	.4	6.3
Dried for 24 hr at 105° C	4.0	.4	3.6
Dried for 24 hr at 70° C in circulated air	7.0	.4	6.6
Dried, under vacuum, on steam drum	2.7	.3	2.4
<i>Commercial product</i>			
Sample A	3.2	0.4	2.8
Sample B	6.4	2.0	4.4

been proposed by the Association of American Feed Control Officials as a required level for a commercial feed supplement. If the minimum values are considered (Table 1, col. 4), the amount of vitamin B₁₂ in the samples dried under mild conditions was well above that required. A commercially available dried sludge contains similar significant amounts of vitamin B₁₂.

The existence of such a large potential supply of vitamin B₁₂ in activated sludge is important, for dried sludge has heretofore been of value only as a fertilizer. Chick feeding tests are now being conducted at Beltsville, Maryland, by Herbert R. Bird, Bureau of Animal Industry, U. S. Department of Agriculture.

References

1. SKEGGS, H. R., *et al.* *J. Biol. Chem.*, **184**, 211 (1950).
2. HOFFMAN, C. E., *et al.* *Ibid.*, **181**, 635 (1949).
3. YACOWITZ, H., NORRIS, L. C., and HEUSER, G. F. *Proc. Soc. Exptl. Biol. Med.*, **71**, 387 (1949).